REMARKS

Claims 1 and 3-27 are pending in the application. Claim 2 has been cancelled. Claims 3 and 26 have been amended by the above amendment. Support for the amendments can be found in the original claims as well as throughout the specification. No new matter has been added.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 2-6 and 26 are rejected as being indefinite because of the language "preferably first."

Claim 2 has been cancelled. Claims 3 and 26 have been amended to remove the word "preferably" thereby overcoming the rejection.

Rejections Under 35 U.S.C. § 103(a)

The subject matter of the various claims was commonly owned at the time the inventions covered in the application were made.

Claims 1-12, 15-22, 25 and 27 are rejected under 35 U.S.C. §103 as being unpatentable over Minshull et al. (5,837,458). The Examiner provides the following basis for the rejection:

> As to the limitations of claims 1-12, 15-22, 25 and 27, Minshull et al. disclose a method of evolving a biocatalytic activity of cell. The method applies homologous recombination technique to produce a library of recombinant genes in which the segment differs from each other in at least two nucleotides (See column 2, lines 40-45.) The method involves in Vivo formats plasmid-plasmid recombination. The initial substrates for recombination are a collection of polynucleotides comprising variant forms of a gene and the variant forms show substantial sequence identity to each other to allow homologous recombination between substrates (See column 7, lines 53 to column 8, lines 1-20). Plasmid containing diverse substrates are initially introduced into cells by any method (As recited in claims 1-12, 15-22, 25 and 27). In general, any type of cells can be used as a recipient of evolved gene, for example bacterial cells and yeast (See column 13, lines 31-46) (as recited in claims 7-8).
>
> One of ordinary skill in the art would have been motivated to apply the method of Minshull et al., because the method of Minshull et al. involves homologous recombination technic to produce a DNA library as disclosed and in which the recursive sequence recombination technique can be used for the host

> determination genes to be modified to grow on the new host in greater growth rates (See column 34, lines 58 and column 35, lines 1-8). Constructing a kit including all components needed to perform a method was well known in the art at the time of the instant invention. It would have been prima facie obvious to carry out the method as claimed.

Claim 2 has been cancelled. This rejection is respectfully traversed with regard to the pending claims. The present claims are drawn, *inter alia*, to methods of constructing a DNA library *in vivo*. The method is essentially as shown in the diagram on page 12 of this response. The method includes introducing, into each of a plurality of host cells, (i) at least one vector molecule having a first region (R1) and a second region (R2), and (ii) at least one nucleic acid insert having a first region homologous with said first region of the vector, a library encoding element (L1 and L2), and a second region homologous with said second region of the vector, and allowing homologous recombination and gap repair between the vector and the nucleic acid insert to occur, generating a plurality of vector molecules from the host cells, each vector having a different nucleic acid insert. This results in a DNA library, i.e., plurality of different library encoding elements (L1 and L2) inserted into the vector molecules. Claim 27 is drawn to a kit allowing the interchangeable use of a DNA library in more than one application comprising the recited PCR primers.

Minshull et al does not teach, suggest or provide a motivation to arrive at the claimed methods. The present claims all recite a method of constructing a DNA library (or a kit for use of a DNA library) that requires homologous recombination between first and second regions of a **vector** that are homologous to first and second regions flanking a library element encoding region in an **insert** molecule. The only place where Minshull mentions any in vivo format of homologous recombination is at 7:53 *et seq.*, where Minshull provides as follows.

> 2) In Vivo Formats
> (a) Plasmid-Plasmid Recombination
> The initial substrates for recombination are a collection of polynucleotides comprising variant forms of a gene. The variant forms usually show substantial sequence identity to each other sufficient to allow homologous recombination between substrates.
> ***

> There should be at least sufficient diversity between substrates that recombination can generate more diverse products than there are starting materials. There must be at least two substrates differing in at least two positions.
> ***
> The diverse substrates are incorporated into plasmids.
> ***
> Having introduced the plasmids into cells, recombination between substrates to generate recombinant genes occurs within cells containing multiple different plasmids merely by propagating the cells. However, cells that receive only one plasmid are unable to participate in recombination. (all emphasis added).

As provided by Minshull as quoted above (and as diagramed in the figure enclosed herewith), the Minshull method is a method of generating more diverse forms of a particular gene sequence. In the Minshull method, homologous recombination occurs between at least two variant forms of a gene differing in at least two positions, each variant form contained in a plasmid, in order to provide even more variant forms of the gene (see the figure enclosed herewith illustrating the Minshull in vivo method). This is why "cells that receive only one plasmid are unable to participate in recombination." (That is, if there is only one plasmid comprising a variant form of a gene, there is nothing for that gene sequence to recombine with). This is in contrast to the presently claimed methods, in which homologous recombination occurs not between two gene sequences in two plasmids, but rather between a plasmid (or other vector) and an insert molecule, specifically between first and second regions of a vector that are homologous to first and second regions flanking a library element encoding region in an insert molecule, as required by the claimed methods. The homologous recombination of Minshull results in cells bearing plasmids containing variant ("evolved") forms of a particular gene sequence. In contrast, as explicitly recited in independent claims 1 and 3, homologous recombination in the present methods results in "a plurality of vector molecules . . ., each vector molecule comprising a different insert molecule." The differences between the two methods can clearly be seen in the figure enclosed herewith.

Thus, Minshull discloses homologous recombination in vivo between two genes, each contained in a plasmid, in order to diversify ("evolve") a gene sequence. This is a completely different method than the presently claimed methods of making a DNA library. There is absolutely no suggestion or motivation (much less a reasonable expectation of success) provided

by Minshull to use in vivo homologous recombination between any sequences that are not variant forms of a gene. There certainly is no suggestion or motivation to use in vivo homologous recombination between first and second regions of a vector that are homologous to first and second regions flanking a library element encoding region of an insert in order to make a DNA library, or of kits for making such a library. Accordingly, a prima facie case of obviousness for the claimed methods has not been established. The Examiner is respectfully requested to reconsider and withdraw this rejection.

In a further aspect of this rejection, claims 13-14 are rejected as unpatentable over Minshull et al. in view of Fraser et al. (U.S. Patent No. 4,870,023). This rejection is respectfully traversed. Claims 13-14 are drawn to methods of constructing a DNA library in vivo, by generating a plurality of nucleic acid inserts having the recited structure; introducing into a plurality of host cells a vector having a first and a second region and the aforesaid nucleic acid insert, and allowing homologous recombination and gap repair between the vector and the nucleic acid insert to occur.

As discussed above, and as can be seen in the enclosed figure, the primary reference Minshall et al. is directed to a completely different method than the one presently claimed. There is no teaching or suggestion in Minshall et al. of methods for constructing an in vivo DNA library, as presently claimed. Fraser et al. fail to make up for the deficiencies in the Minshall et al. reference. The Fraser et al. reference discloses a recombinant baculovirus construct which encodes fusion polyhedrin proteins capable of forming occlusion bodies containing foreign peptides. The recombinant baculoviruses are formed by insertion into or replacement of regions of the polyhedrin gene that are not essential for occlusion body formation with exogenous DNA fragments. There is no teaching or suggestion in Minshall et al. or the Fraser et al. reference, alone or in combination, describing the vector/insert combination recited by the present claims and its use to generate an in vivo DNA library. Therefore, Applicants respectfully request that this rejection be withdrawn.

In another aspect of this rejection, claims 23-24 and 26 are rejected as being unpatentable over Minshall et al. in view of Liu et al. (U.S. 5,928,868). Applicants respectfully traverse this rejection. As shown above, the primary reference of Minshall et al. does not teach or suggest the vector/insert combination used to generate the DNA libraries in vivo required by the claims. Lieu et al. does not make up for the teachings missing in Minshall et al. Lieu et al. is directed to a three-hybrid system for identifying protein targets to which small molecules bind. The combination of Minshall et al. and Liu et al. fails to provide the elements required by the claimed methods. Moreover, the hybrid system described by Liu et al. is an assay for small molecule screening, as opposed to a method for identifying interacting proteins expressed by the in vivo generated libraries. Further, even if the all the elements of the claimed methods were present in the combination of these references (which they are not), there is no motivation in the references cited to combine the homologous recombination system disclosed by Minshall et al. with the small molecule screening/two hybrid assay described by Liu et al. Therefore, Applicants respectfully request that this aspect of the rejection be withdrawn.

In view of the foregoing claim amendments and remarks, it is respectfully submitted that the application is in condition for allowance.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a Petition for Extension of Time with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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Version with markings to show changes made

In the claims:

Claim 2 has been cancelled.

Claims 3 and 26 have been amended as follows:

3. (Twice Amended) A method of constructing a DNA library, comprising:

providing a plurality of nucleic acid molecules wherein each of said nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to the first common sequence of the nucleic acid molecule and having a second region which does not hybridize to said first [(and preferably] or second[)] common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to the second common sequence of the nucleic acid molecule and having a second region which does not hybridize to said second [(and preferably] or first[)] common sequence;

forming a reaction mixture which includes said plurality of nucleic acid molecules, said plurality of said first primers, and said plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of said first primer/said first common region/a library element encoding region/said second common region/a second region of said second primer;

providing a plurality of host cells;

providing a vector molecule having a first region which is homologous with said second region of said first primer, and a second region which is homologous with said second region of said second primer;

introducing said vector molecule into each host cell of the plurality of host cells; and

introducing one or more of nucleic acid insert molecules from said plurality of nucleic acid insert molecules into each host cell of the plurality of host cells,

generating a plurality of vector molecules from said plurality of host cells, each vector molecule comprising a different nucleic acid insert molecule,

thereby providing a DNA library.

26. (Twice Amended) A method of constructing a DNA library for screening in a two-hybrid system, comprising:

providing a plurality of nucleic acid molecules, wherein each of the plurality of nucleic acid molecules includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to said first common sequence of said nucleic acid molecule and having a second region which does not hybridize to said first [(and preferably] or second[)] common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to said second common sequence of said nucleic acid molecule and having a second region which does not hybridize to said second [(and preferably] or first[)] common sequence;

forming a reaction mixture which includes the plurality of nucleic acid molecules, the plurality of said first primers, and the plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of the first primer/the first common region/a library element encoding region/the second common region/a second region of the second primer;

providing a plurality of host cells;

providing a vector having a first region which is homologous with the second region of the first primer, and a second region which is homologous with the second region of the second primer, wherein said vector further includes a transcription factor activation domain;

introducing a vector molecule into each of each host cell of said plurality of host cells;

introducing one or more of the nucleic acid insert molecules into each host cell of said plurality of host cells under conditions which allow for recombination and gap repair to occur;

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein includes a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by the DNA-binding domain and wherein said detectable gene expresses a detectable protein when the test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media; and

selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.